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# The Use of Plug Systems in the Micropropagation of *Amelanchier Alnifolia*

Conducted by
Prairie Plant Systems Inc.

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# THE USE OF PLUG SYSTEMS IN THE MICROPROPAGATION OF AMELANCHIER ALNIFOLIA

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# 1. INTRODUCTION

The saskatoon berry (Amelanchier alnifolia)is a berry crop belonging to the North American prairie area. The berries have been harvested from wild plantings ever since the prairies have been inhabited and in recent years there has been more interest in developing this crop as a commercial fruit crop.

The saskatoon berries are used as fresh, frozen or preserved fruit, jams, wine, pie-filling and other baked desserts (Mazza,1979). The fruit has attracted consumer attention through its color, flavor and appearance. Currently, commercial saskatoon plantings occupy several hundred acres in both Saskatchewan and Alberta but researchers say that orchards of saskatoon berries could take root on as many as 10,000 acres of prairie soil in the next few years (Davidson,1987). There is considerable interest among growers to expand and develop new plantings with a total potential market for sales of 100,000 plants per year in Canada and the USA. However, the problems with expanding commercial plantings are the cost of grafted and budded nursery stock and the lack of uniformity of plants when propagated as seedlings.

Asexual propagation of saskatoons has the advantages of preserving the identity of the selected cultivar thus providing uniform flowering, fruit set,ripening, size, quality and quantity of fruit. This method also speeds the time to fruit production over seedlings (Hartmann et al.,1990). Labor requirements for asexual propagation are usually high,so rooting percentages of 50% or more are required for asexual propagation to be economically viable (Cumming,1976).

There are several methods used to asexually propagate A. alnifolia. Budding and grafting of a desired clone onto an established rootstock has been tried with limited success. Root cuttings placed in a mist bed are also used but results have been inconsistent (Nelson,1987). Bishop and Nelson (1980) worked with root cuttings, hardwood cuttings, leaf bud cuttings and softwood cuttings and stated that saskatoon cuttings formed roots poorly and developed little vegetative growth. The difficulty of bringing these rooted cuttings through the first winter was also a major obstacle to commercial propagation.

As a result of the difficulties in producing sufficient plants to meet the demand for A. alnifolia using common propagation methods, attention is now being turned toward using in vitro micropropagation techniques (Friesen,1988). In a 1980 symposium held in Edmonton, Alberta it was concluded that one of the major factors limiting the advancement of the saskatoon industry was the inability to propagate the large-fruited, more productive cultivars in quantities to meet the demand for plants at an economical cost. It was also stated that the immediate future of the industry will have to depend on the less desirable seedling forms (Nelson,1987). Although the production of in vitro plants has been reported, the protocol for their production procedures have not been released by commercial labs involved (George and Sherrington,1984).

This paper will briefly review micropropagation in general and will specifically investigate the different methods used to root out plantlets after they have been removed from culture media.

## 2. MICROPROPAGATION DEFINED

In vitro culture involves growing or maintaining plant tissue or organized plant parts on an aseptic, artificial media which supplies the nutrient requirements of the plant (George and Sherrington,1984).

Micropropagation is the application of aseptic in vitro culture techniques towards the propagation of plants from small plant parts, tissues or cells with the aid of plant growth regulators (George and Sherrington,1984). Where organized plant parts are aseptically grown in culture, the term "organ culture" is more appropriate. Organ culture includes use of meristem or shoot-tip culture, single node and embryo culture, leaf primordia, immature flowers and fruit.

Some reasons for micropropagation of horticultural crops include:

a) the production of pathogen free plants and the maintenance of a nuclear stock of healthy plants
b) rapid increase of selected genotypes for breeding purposes
c) large scale production of new cultivars for rapid introduction into commercial markets
d) propagation of plants for which traditional methods are difficult, impossible or unprofitable
e) reduction in number of nursery stock plants
f) importation and exportation of plant material thus easing passage through quarantine

(Bhojwani and Razadan,1983:Callaghan,1985)

Some of the disadvantages in the use of micropropagation are:

a) undesired genotypic variation as a result of callus culture
b) high capital costs
c) lengthy research and development of methodology for each cultivar
d) requirement of skilled staff
e) expensive plants produced as compared to traditionally propagated plants
f) small plant size
g) tender plants requiring slow transition to natural environment

(Bhojwani and Razdan,1983; Callaghan,1985)

## 3. STEPS IN THE MICROPROPAGATION PROTOCOL

Micropropagation requires several steps beginning with the selection of the desired plant tissue source and continuing through to the establishment of the plantlet in the greenhouse or in the field. These steps have been summarized and presented according to the outline given by George and Sherrington (1984).

### 3.1 Stage 0: Mother Plant Selection and Preparation

The desired plant is selected and treated to reduce contamination and to improve response to future in vitro treatments. The pretreatment may include osmotic adjustment in the tissue, improving its nutrient condition, altering photoperiod or heat treatment to kill viral infections.

### 3.2 Stage 1: Establishment of an Aseptic Culture

This step includes the selection of plant tissue, surface sterilization of the tissue and the induction of shoot growth. The plant tissue chosen depends on the method of multiplication to be used and the nature of the plant. Generally, actively growing shoots give the best results (Hartmann et al.,1990).

Initial washing under running water is generally recommended and then the tissue is sterilized with any of a number of agents such as,sodium hypochlorite (bleach), ethyl alcohol or hydrogen peroxide. Sterilization is often followed by several rinses in sterile distilled water and all stages subsequent to this require a sterile environment.

In order to induce the growth of shoots or callus the plant tissue must be placed onto a culture medium. This medium may be solid, semi-solid of liquid with additions of plant growth hormones suitable for the desired growth habit. Generally 4-6 weeks are required to initiate substantial growth of the plant tissue on the culture.

### 3.3 Stage 2: The Production (Proliferation) of Suitable Propagules

Proliferation includes the multiplication of organs or structures on the media which can then in turn be cultured to produce more intact plants. These propagules may arise from axillary or adventitious shoots of other tissues of the plant being cultured. The media used and the plant tissue used will ultimately determine the resulting rate of multiplication. Propagules are usually recultured starting with stage 2 in order to increase the number of plants. The result of this process is exponential multiplication of the original plant that facilitates the production of thousands of identical plants per year.

### 3.4 Stage 3: Preparation for Growth in the Natural Environment

Propagules need to be prepared to leave the culture media and to be transplanted into a more natural environment. The change from an heterotrophic lifestyle to an autotrophic habit requires the formation of roots. Root formation is a complex process which may require special treatments to prepare the tissue for this process. Rooting of the shoots may take place either in vitro or ex vitro (George and Sherrington,1984). In general, root formation requires treatments that lower inorganic salts and increase the available supply of auxins (Bhojwani and Razdan,1983). Shoots are quite tender and heterotrophic to begin with and are usually kept under conditions of high humidity in a protected environment. The rooting substrate is often a mixture of two or more of the following substances: peat, vermiculite, perlite, rockwool, pumice, sand or soil with or without the addition of auxins (George and Sherrington,1984).

### 3.5 Stage 4: Transfer to the Natural Environment

Plantlets which have formed roots in either stage 2 of 3 can now be placed in a loose, well drained medium for continued root formation and hardening off.

Since plants growing in culture medium are used to very favorable conditions and are usually heterotrophic, their leaves are often very thin, have poor epicuticular wax, poorly functioning stomata and their photosynthetic systems are not well developed. Therefore, plantlets are often subject to dehydration and require a period of adjustment to high light intensities and low relative humidity (Ziv,1986). A gradual change to natural conditions through a slow lowering of humidity over 10 -15 days and by shading from strong light for some time is a successful acclimation procedure with most plants.

A common problem with many plants in micropropagation is the onset of dormancy during rooting which greatly slows the growth of plantlets This is especially a problem with A. alnifolia. This condition can be overcome through application of gibberellins or through a cold treatment of 0 to 10 degrees C for up to 2 months in order to break dormancy. (Hartmann et al.,1990).

## 4. VARIOUS CONTAINER SYSTEMS USED IN ROOTING OF MICROPROPAGATED PLANTS

New and different types of containers for propagation of plantlets are continually being developed with the main goal of reduced handling costs. Direct sticking of cuttings or explants into small containers is now being used as compared with direct sticking into conventional propagation trays (Hartmann et al.,1990). Direct sticking into small containers saves production steps and avoids root disturbance of plantlets which can lead to transplant shock.

### 4.1 Flats

Flats are shallow plastic, wooden, or metal trays with drainage holes in the bottom but with no divisions present in the tray. These trays are used for germinating seeds or rooting cuttings, since they allow plantlets to be moved around easily if necessary. Rot resistant woods such as cedar or redwood are preferable for flats. Galvanized steel or plastic flats are available in various sizes and they have the advantage of nesting or stacking which reduces storage space requirements (Hartmann et al.,1990). The problem with flats for rooting out cuttings is that root disturbance often occurs when the plantlet is removed from the flat to be placed in a larger container.

## 4.2 Plastic Containers (Pots)

Plastic containers, round and square, are commonly used because of several advantages. They are non-porous, reusable,lightweight and require little storage space because they will nest. Polyethylene containers are sturdy yet flexible but cannot be steam sterilized. However, most common plant pathogens can be controlled by a hot water washing (70 C) and a rinse in sodium hypochlorite.

A type of plastic container, commonly used in reforestation projects, are rootrainers. Rootrainers are made of preformed, hinged plastic sheets which fold together and lock to form a set of four smaller containers which fit into a special plastic holding tray. One plantlet is grown in each small sub-container and the vertical groves on the sides of the containers reduce the chance of root spiralling. The hinged container can be opened to inspect the roots or to remove the plant with minimal root disturbance (Hartmann et al.,1990).

## 4.3 Fibre Pots

Containers of pressed peat and wood fibre are available in various shapes and sizes. These containers are biodegradable and are usually set into the soil along with the plant. Peat pots are best used when the plant is to be held in them only a short time and then placed in a larger container or into the field (Hartmann et al.,1990). The advantage of the peat pots is that they can be placed right into the soil, thus saving transplanting time and avoiding any root disturbance. However, small peat pots with plants growing in them for any length of time will often fall apart when moved because of the constant moisture present in them. In addition, plantlet roots often grow in an undesirable spiral pattern in this type of rooting container.

## 4.4 Peat and Fibre Pellets

Blocks of pre-formed solid material, with a pre-formed hole or slit have gained attention as a rooting medium for cuttings. Fertilizers are often included in the material as well. A common example, the Jiffy peat pellets (Jiffy Products of America Inc.), are made of highly compressed sphagnum peat. When water is added, the peat pellet swells to several times its original size but is contained and held together inside a nylon, netlike structure. The peat is soft enough for a cutting or seed to be inserted directly into it. The peat package becomes a part of the plant unit and is transplanted into the soil along with the plant which replaces the need for a pot and a soil mixture for propagation (Hartmann et al.,1990).

### 4.5 Synthetic Plug Systems

Synthetic plugs or rooting blocks are becoming more common in the nursery industry because of their suitability for automation. One horticulturalist stated that the development of the seedling plug is one of the major advances in the bedding plant industry in the last decade (Hartnett,1985). A plug is a small cylinder of artificial rooting medium which is contained in a small cell or cup. Up to several hundred plug cells are formed into a single sheet of polystyrene,styrofoam of other suitable material. After the seed, cutting or micropropagated material has been placed in the plug, the plug is placed in the plug tray. The plug tray is then placed in a greenhouse or rooting chamber and is provided with moisture through mist, fog or capillary mats and is lightly fertilized at intervals (Hartmann et al.,1990).

There are several types of plugs available. One type of plug is simply formed in a tube-like cup from a pre-mix of peat, sand, perlite and vermiculite. This type of plug is not solid or stabilized and relies on the root mass of the plantlet to form a solid plug suitable for transplanting (McCown,1986). Commercial plug systems for microcutting propagation are available from several companies. Techniculture plugs (Castle and Cooke Techniculture Inc.: Salinas,CA) are a stable mixture of peat moss and hydrophillic latex binder. These plugs contain no nutrients but do have a relatively good cation exchange capacity plus the media will not break up when transplanted.

Oasis Horticubes are another type of commercial plug (Smithers-Oasis, Kent, OH). These plugs are a polyurethane, open-cell, foam cube which has no cation exchange capacity, no buffering capacity and contains no nutrients. The horticube is made of hydophobic material and therefore water is held in the cube by capillary action which limits the size of the cube which can be used (McCown,1986).

## 5. FEATURES OF A PLUG SYSTEM FOR MICROPROPAGATION

### 5.1 Desired Characteristics of a Plug System

If plugs are to become a major component of a micropropagation system, then they will most likely serve as a rooting media as well as the environment for growth and preparation for transplanting of the plantlet (McCown,1986). Whether or not a plug system is useful depends on how closely it fits the characteristics which are necessary for this important stage in the micropropagation process. The primary goals of using a system such as plugs are to increase the efficiency of transition from the laboratory to the growth environment as well as to increase the standardization of the micropropagated product (McCown,1986).

The medium used for propagation by plugs must provide good drainage, and water holding capacity. It must also have enough nutrient holding capacity to keep the cuttings actively growing while rooting. The plug should provide a moisture/air relationship of 50/50 by volume plus the medium should be uniform, reproducible and stable. However, all of these qualities should be achievable with a minimum of set-up time and maintenance (Deppe, 1985).

The plug media should be non-toxic because microcuttings are very sensitive to toxins. Low toxicity in plugs and peat pellets is not always easily achieved and has been a large problem in using plugs for micropropagation (Maginnes,1990). Plugs should also be free of insects and pathogens because the tender microcuttings are very susceptible to disease (McCown,1986). This susceptibility is increased because the plugs are held in warm, humid environments for lengthy periods during rooting.

Since the insertion of the microcutting or seed into the plug is a hand operation, it is labor and cost intensive. Therefore it is important that microcuttings are easily inserted into the plug and that potential exists for mechanization of this step (McCown,1990).

Plug trays are available in a variety of sizes and shapes. The trays which hold larger plugs are generally easier to grow but are less efficient in terms of space utilization. Small celled trays are space efficient but harder to grow plants in. The size of the plug also effects the nutrient and moisture buffering capacity of the medium. Generally, a larger plug has a larger buffering capacity (Welsh,1984). However, efficient space utilization is important because microcuttings generally require an expensive physical environment for rooting and acclimation (McCown,1986). One unit of agar culture space will require 2-3 units of rooting space, Therefore, even in small micropropagation operations, space efficiency should be considered. Weight of the plug trays is also a factor because many of the plants will likely be shipped long distances.

## 5.2 Advantages of Plug Systems

Several nurserymen have commented on the advantages of working with plug systems. Deppe (1985) stated that the plug system provided his customers with the most uniform plant possible. He felt the plug system he was using was clean and neat, easy to work with, easy to mechanize and allowed for maximum production from unskilled labor.

Welsh (1984) used a plug system in New Zealand and stated that it was an efficient means of producing seedlings for the commercial market. He felt that using plug trays reduced the spread of damping off diseases because each plant was isolated in its own compartment. He also felt that the transplanting time from the plug to the field or larger containers was reduced by at least 50%. In addition, transplant shock to the young seedling was avoided using the plug system. Furthermore, valuable heated greenhouse space was used more efficiently with plugs and therefore production levels could be increased using the existing resources of the company.

McCown (1986) stated that one major advantage of any plug system is the ease of transfering a single plant to a new container. There is much less root injury and subsequent transplant shock when transplanting plugs in contrast to transplanting bed-grown material where root injury cannot be avoided. Other advantages seen by McCown (1986) were that a pre-made plug system offered the micropropagater a standardized product which could be used directly in the laboratory, shipped readily and would be easily used by the receiver. Ease of shipping also includes the idea that some of the commercial plugs contain no soil and are therefore easily transported across international borders (Grow-Tech Inc.; Watsonville,CA).

A requirement for any system that seeks to be applicable to large industries is that the system must be able to be mechanized. Mechanized transplanting of plugs to the field or larger containers has been accomplished for some plug systems which is seen as a large advantage for this system (Blackmore Company: Belville, MI).

With certain preformed plugs, the plug media contains a non-toxic glue and therefore it is not necessary for the roots of the plantlet to hold the media together. Therefore, plants can be transplanted earlier than plants in loose media or in bed propagation systems. This is an advantage because it allows a greater flow of plants through the greenhouse per year (Techniculture Plug Systems; Salinas,CA).

## 5.3 Disadvantages of Plug Systems

Welsh (1984) stated that the use of plugs had the disadvantages of requiring greater skill for production and higher risks involved with the new techniques. This opinion is not shared by all growers however.

Harnett (1995) felt that one of the problems with plugs was the complex watering requirements of the system. Intermittent mist systems along with capillary mats are probably the most widely used watering system. Because of the small size of individual cells, irrigation of plugs trays must be both frequent and accurate in distribution (Welsh,1984). However, mists are often uneven or leave the plugs too wet.

The Techniculture plug company stated that water and humidity are the most critical aspects of a plug system. Under mist, the plugs remain too wet if allowed to drip drain. They also felt that capillary mats and fog systems were best in order to keep the moisture levels in the plug uniform and constant. However, they also mentioned one of the large disadvantages of the plug system is that it has a very low buffering capacity against low humidity conditions. Even a short drying period of 1 hour can harm a plant in a plug since the plug shrinks slightly upon drying and pulls away from the plug tray exposing the roots to air.

Probably the most widely used type of plug is a plug that is individually designed and filled to meet the requirements of a specific nursery's needs. The nursery/laboratory will often premix its own media and fill the plug trays with this media. As already stated, this type of plug is not solid so it relies on the plant roots to make the plug solid enough for transplanting. This requirement however, places a small window on the time when the crop can be transplanted or shipped (McCown,1986). If the plant is not rooted well enough the plug is easily damaged during transplanting. However, plants are often quite large by the time they have sufficient rooting to form a solid plug and at this stage the plant has a very short life span left in the plug.

Many microcuttings such as A. alnifolia and other woody plants are somewhat hard to root (Bishop and Nelson,1980). These type of plants often require a larger plug but in order to maintain space efficiency yet increase rooting volume, a deeper, small diameter plug is often used. However, a small diameter, deep plug is difficult to fill uniformly with medium and is hard to remove from the container (McCown,1986). Because a small diameter, deep plug is difficult to use, many woody plant propagators have gone to using larger diameter plugs or rootrainer plastic containers (Hartmann et al., 1990).

Another large disadvantage of premade plugs or peat pellets are problems with toxicity. Peat pellets produced by some companies have been found to be high in salts which adversely affects seedling or microcutting growth (Maginess,1990). Wheeler et al. (1985) reported that polyurethane foam plugs can be quite toxic to plants, particularly to small seedlings. The injury from the plug typically occurs on stem tissue which is in direct contact with the foam. The toxicity problem often shows up as a brown discoloration on the stem tissue. The discoloration can result in complete collapse of stem tissue and eventual death of the plantlet.

Wheeler et al. (1985) felt that the toxicity problem was due to amine or isocyanate compounds trapped in the air cells of the polyurethane during foam production. They recommended that polyurethane foam plugs used in plant propagation either be rinsed in ethanol and distilled water or be heated to 100 C for 2 hours before use, to remove all toxic and inhibitory factors. This treatment would apply to such products as the Oasis horticubes.

## 6. ADDITIONAL ELEMENTS OF PLUG SYSTEMS

### 6.1 Explant Quality in Plug Systems

The quality of the explant coming out of culture is important to rooting success. With many species, there is a close relationship between the size of the microcutting and the success of rooting. Healthy and well developed leaves are also important on the explant being placed in the plug. Ideally, microcuttings should be actively growing at the time of sticking and should remain growing through the rooting period. However, when working with woody species, they often stop growing during the rooting phase and do not easily begin growth again but remain in as extended dormant period (McCown, 1990).

## 6.2 Rooting Environment in Plug Systems

Neal (1989) reported of a greenhouse owner who used plugs to grow bedding plants from seed. The toughest part of plug growing for this greenhouse manager/owner was successful seed germination. Successful seed germination has several parallels to successful rooting of plugs. Both operations are difficult and require controlled conditions.

Neal (1989) states that successful seed germination in plugs was brought about most successfully by the use of a "sweat chamber". The chamber was used for several reasons which apply to rooting out explants. 1) The chamber reduced the amount of space required by the plug trays because trays could be set on shelves instead of on valuable bench space in the greenhouse. When explants are freshly placed in plugs they must be shaded from intense light. Therefore, artificial light between the shelves is often sufficient in the sweat chamber.

2) The sweat chamber may reduce the time from placement of the microcutting into the plug to the time when it is successfully rooted and ready to go into the greenhouse or be transplanted.

3) The most important aspect of the chamber is the efficiency with which the environment can be controlled. Humidity is the critical factor in rooting and therefore it is crucial that microcuttings not dry out during this stage (McCown, 1986).

McCown (1986) also refers to several systems used to control the environment when rooting cuttings in plugs. Plug trays can be put into small rooting chambers the size of one tray. A capillary mat is used beneath the plug trays to provide even moisture distribution. On a slightly larger scale, a sweat box the size of the greenhouse bench might be used. A third concept is a fog system which would maintain high humidity (above 80%) through the entire greenhouse. It is interesting to note that McCown (1986) also states that mist systems are not desirable for rooting because the on/off cycle either keeps the plugs too wet or allows the microcuttings to dry out.

The temperature for the rooting chamber is often kept between 21-27 C. However, it is important that the temperature of the plug and roots be a few degrees higher than the air temperature because this increases the humidity around the microcuttings (Techniculture Plug Systems). The Techniculture plug company recommends a fertilization regime that would begin with 60ppm each of N,P and K. Fertilization should be started early (emergence of first true leaf for seedlings) and is most easily applied along with the watering system.

## 6.3 Storage of Explants Rooted in Plugs

Some species of plants when rooted in plugs, have been stored at 5 C under low light for at least 30 weeks with no damaging effects (McCown,1986). The ability to store the rooted explants in plugs is very beneficial for a micropropagation laboratory. By using this technique an inventory can be built up in the slow seasons and production can continue all year long. Since plugs are space efficient and cold storage space is expensive, the plug system would provide an economic storage system.

### 6.4 Performance of Plugs Used in the Field

Studies at the university of Wisconsin recorded the success of direct planting of rooted plugs into the field using several different plant species such as: Betula, Ulmus, and Solanum. Survival of plants rooted in plugs was high (90%) provided:
a) the plantlets were of high quality
b) the field was properly prepared so that good plug-soil contact was achieved
c) irrigation was available in the first 7 days
d) the top of the plug was buried in the soil to prevent it from drying out.
If these conditions were present, root penetration into the soil could be seen within a few days of planting (McCown,1986).

### 6.5 Problems with Amelanchier in plug systems

McCown (1986) reported that Amelanchier cuttings produced roots readily in Techniculture plugs but the roots deteriorated as they reached the bottom of the plug. He also observed that the roots grew mainly on the outside of the plug rather than through the plug medium. He was not able to determine if the problem with the plug was a toxin in the plug or was with the physical characteristics of the plug.

## 7. EXPERIMENTS WITH ROOTING Amelanchier alnifolia IN PLUGS

Several experiments involving the rooting out of micropropagated Amelanchier alnifolia were conducted by Prairie Plant Systems of Saskatoon, Saskatchewan. The following is a brief report on the methods and findings of these experiments

### 7.1 Objectives of Experiment Type A

These experiments were conducted to investigate the rooting performance of explants of 5 different A. alnifolia cultivars in Techniculture plugs versus rootrainer (hillson) trays containing a potting mixture. It has been reported that saskatoon cuttings do not root well and that they root especially poorly in preformed, synthetic plugs (McCown,1986). However, since plugs offer several advantages including the fact that they are space and time efficient and lend themselves to mechanization it would seem profitable to study the use of plugs in the micropropagation of saskatoon explants.

The objectives of these experiments were threefold:
1) to investigate the comparative rooting ability of the cultivars; Honeywood, Thiessen, Martin, Smoky, and Lemonade.
2) to compare overall rooting percentages in Techniculture plugs compared to rootrainer trays
3) to observe the factors affecting rooting and growth of A. alnifolia explants in plugs and rootrainer trays

## 7.2 Materials and Methods

All of the saskatoon cultivars were tissue cultured under identical conditions in an agar media for 8 weeks. At that point explants were chosen which were 1.5-3.0 cm tall. Callus growth was removed from the explants and they were then placed either into Techniculture plugs or into rootrainer trays.

Rootrainers trays were filled with a soil mixture consisting of 2 parts peat, 1 part perlite and 1 part vermiculite. Both the plugs and soil mixture were drenched with a solution of distilled water containing 0.25 g/l 20-20-20 fertilizer. The solution also contained a concentration of 2.45 micromolar indole butyric acid (IBA) to stimulate rooting. Both the plugs and soil mixture were drenched the day before the explants were placed into them.

After the explants had been inserted into the plugs and rootrainers, they were immediately placed into a rooting chamber. Within the rooting chamber plants were watered daily with distilled water for the first week and thereafter they were monitored daily and watered every second day. Fertilizer solution (0.25g/l 20-20-20) was also added once every week. Artificial lighting was supplied in the rooting chamber on a 16 hour photoperiod at an intensity of 93 mE/m2/sec.

After 4 weeks in the rooting chamber the plug trays and rootrainers were transferred into a mist bed in the greenhouse. After another 4 weeks in the mist bed, a rooting percentage count was taken. Rooting of explants was assumed to have occurred when secondary growth appeared on the explant.

## 7.3 Results

| Cultivar | Rooting Percentage | |
|---|---|---|
| | Rootrainer trays | Plugs |
| Thiessen | 42% | 5% |
| Smoky | 38% | 0% |
| Martin | 36% | 3% |
| Honeywood | 16% | 0% |
| Lemonade | 3% | 10% |
| Overall | 30% | 3% |

Within the rootrainer trays the Thiessen cultivar was able to achieve the highest rooting percentage (42%). Smoky and Martin showed similar rooting at 38% and 36% respectively. However, Honeywood and Lemonade both showed extremely low levels of rooting. In the plugs, Lemonade showed the highest rooting percentage at 10% with all other cultivars being at or below 5%. Overall, the average rooting percentage of all saskatoon cultivars in the rootrainers was 30% and in the plugs was only 3%.

The saskatoons that were able to root in the plugs were much smaller and more chlorotic than the plants in the rootrainers. The leaves of the plants in the plugs showed much higher interveinal chlorosis and had much smaller leaves (1 cm wide X 1.5 cm long) compared to leaves of plants in the rootrainers (5 cm wide X 6 cm long). The roots of the saskatoons in the plugs were also stunted and thickened as compared to roots in the rootrainer trays.

It was noted that fungal hyphae developed on the plugs despite a single autoclaving. Algal build up was also noted on both the plugs and rootrainers.

## 7.4 Discussion

Cumming (1976) stated that 50% rooting was necessary to make commercial propagation of softwood cuttings economical. Therefore, in these experiments, neither the rootrainers or plugs would be considered an economical means for rooting microcuttings of saskatoons. However, several observations were made which may have contributed to poor rooting percentages.

The Techniculture plugs did not provide ideal surface contact with the explant and they did not hold as much water or nutrient solution as the potting mix. The surface of the plug dried out sooner than the potting soil in the rootrainers possibly due to drainage within the plug itself. As a result of the less favorable and fluctuating moisture status in the plugs, the plants wilted and browned off more rapidly in the plug trays than in the rootrainer trays. These conditions were likely a major cause of lower rooting percentages in the plugs.

In order to decrease fungal growth on the plugs it was recommended that the plugs be double autoclaved before being used. It was also recommended that a capillary mat be placed under the plug tray to decrease drainage and moisture fluctuation in the plugs.

## 7.5 Conclusions

It was concluded that plugs are more convenient and time efficient to work with but were less effective in bringing about rooting as compared rootrainer trays filled with potting mix. Also, explants in plugs were more susceptible to variations in humidity and temperature than explants rooted in rootrainer trays mainly because of differences in rooting media and rooting volume.

Despite the poor rooting percentages seen in both the plugs and rootrainers, performance may be improved by changes in media amendments and environmental conditions during rooting.

## 8. EXPERIMENT TYPE B

### 8.1 Objectives of Experiment Type B

These experiments were conducted to compare rooting of the Thiessen saskatoon berry in Techniculture plugs versus rootrainer (hillson) trays containing a potting mix. This study was intended to further investigate and verify the findings and observations of the type A experiments.

### 8.2 Materials and Methods

All explants were cultured on media for 6 weeks prior to being placed in the rooting media. All explants chosen were very vigorous, 1.5-3.0 cm tall and displayed very few signs of interveinal chlorosis. Explants chosen for this experiment were larger on average than explants used in experiment type A.

The plugs and rootrainer trays were drenched with nutrient solution as in experiment type A except that no IBA was included. Pruski (1987) has shown that Thiessen saskatoons actually root better with no hormone present.

After plugs had been inserted into the rooting media, they were placed in a rooting chamber for 4 weeks. Nutrients, water and light were supplied as in experiment type A. However, the humidity was kept at a higher level than experiment type A throughout the 4 weeks that the plants were in the rooting chamber.

### 8.3 Results

| Cultivar | Rooting percentage | |
|---|---|---|
| | Rootrainers | Plugs |
| Thiessen | 79% | 46% |

After 4 weeks in the rooting chamber, rooting percentages were recorded according to the same criteria as in experiment type A. The rootrainer trays had a much higher rooting percentage (79%) compared to the plugs(46%). The plants in the plugs again displayed more signs of desiccation, stunting, smaller leaves and chlorosis than plants in the rootrainer trays. Most of the rooted plantlets also showed signs of nutrient deficiency.

### 8.4 Discussion and Conclusions

It was again evident in this experiment that rootrainers produced much higher rooting percentages than the plugs. In fact, rootrainers (79% rooting) displayed an economical level of rooting whereas plugs were again not an economical rooting media.

Several factors may have helped to increase rooting of the Thiessen cultivar in this experiment. Increased humidity in the rooting chamber seemed to keep plantlets from desiccating before they were rooted which helped to increase rooting in both systems. In addition, no IBA hormone was used in the nutrient solution and since the cultivar Thiessen roots better with no hormone, we would expect higher rooting percentages. Furthermore, a greater effort was made in this experiment to use larger, healthy explants. Large, healthy explants are known to root much better than smaller plants (McCown,1986).

Since a nutrient deficiency was showing up in most of the rooted plantlets, further work to determine the nutrient needs of explants in rooting media was recommended.

## 9. EXPERIMENT TYPE C

### 9.1 Objectives of Experiment Type C

These experiments were conducted to compare explant rooting percentages of the saskatoon cultivar SM-8901 when placed in a potting premix contained in Techniculture plug trays versus rootrainers trays.

### 9.2 Materials and Methods

All explants were cultured on Agar media for 6 weeks prior to being placed in the rooting media. All explants chosen for rooting were 1.5-3.0 cm tall and were very vigorous.

A Sunshine mix #1 potting soil (Fisons Western Corporation; Vancouver,B.C.) was used in both containers and was presoaked with 1/4 strength Murashige and Skoog (1962) macronutrient solution. However, no IBA rooting hormone was added to the rooting media along with the nutrient solution. Soil pH was adjusted using $CaCO_3$ to achieve a pH of 6.8.

Once the explants had been placed in the rooting media, the trays were placed in a rooting chamber. Conditions in the rooting chamber and supply of water, nutrients and light were all similar to experiment type B. After 4 weeks in the rooting chamber and 3 weeks in the mist bed, the plants were observed to see if rooting had taken place.

## 9.3 Results

| Cultivar | Rooting percentage | |
|---|---|---|
| | Rootrainer trays | Plug trays |
| SM-8901 | 76% | 65% |

During the experiment some of the plants developed waterlogging symptoms but these symptoms disappeared when the watering frequency was decreased. It was also observed that some of the explants initiated rooting as soon as 1 week after sticking but other explants were much slower to begin so that rooting percentages increased slowly over a six week period. Some plantlets in the plug trays still showed nutrient deficiencies which may have been the result of a small rooting volume in these rooting containers.

Overall, the variety SM-8901 showed a high level of rooting in both containers. The rootrainers however, still displayed a higher rooting percentage than the plug trays. However, both containers were able to produce rooting levels above the economic cutoff level of 50% rooting.

## 9.4 Discussion and Conclusions

It appears that the rootrainer trays with a larger rooting volume are less sensitive to fluctuations in temperature, moisture and nutrient levels than plug trays as evidenced by a higher rooting percentage in the rootrainer trays.

Plantlets in the plug trays still showed nutrient deficiencies even when growing in the potting premix. Because of this condition, plug trays must be monitored and maintained more carefully than the rootrainers not only in the area of moisture status but also for nutrient status. Since an extended rooting period of 6 weeks is undesirable for laboratory throughput, more work needs to be done on manipulating nutrients, moisture and rooting hormones to an optimum level so that rooting can be accomplished in a shorter period of time and at a more even rate in all plantlets.

It can also be seen that the small rooting volume of the plug trays is not solely responsible for the low rooting percentages in plugs seen in the previous experiments. The use of potting premix in the plug trays greatly increased rooting over the use of synthetic plugs in the plug trays. Therefore, it seems reasonable to assume that the synthetic plug material may be toxic or inhibitory to root growth of saskatoons as noted by McCown (1986).

## 10. EXPERIMENT TYPE C

### 10.1 Objectives of Experiment Type C

These experiments were conducted to investigate the rooting performance of the A. alnifolia cultivar, Martin, when placed in several different rooting media. The main objective of the experiments was to compare rooting percentages of the saskatoon cultivar in rootrainer trays versus Jiffy-7 peat pellets and Techniculture plugs containing a rooting polymer. A second objective was to compare the effect of two different rooting polymers which were used in the Techniculture plugs to enhance rooting.

### 10.2 Materials and Methods

All of the plugs were double autoclaved to reduce fungal growth and damping off which occurred previously in the plugs. In addition, all rootrainer trays, plugs trays and Jiffy containers were washed in a dilute sodium hypochlorite solution. Rootrainer trays were filled with a potting mixture of 2 parts peat, 1 part perlite, 1 part vermiculite. Jiffy-7 peat pellets (Jiffy Products of America Inc.; Chicago, Illinois) made of compressed sphagnum peat, surrounded by nylon netting were also used. Techniculture plugs were included in the experiment but to enhance surface contact between the plug and microcutting a rooting polymer was placed inside the slit in the plug.

Rooting polymer is a granular, organic, absorbent material that provides better water and nutrient utilization by plants. The polymer is reported to increase water holding capacity of the rooting media while improving aeration and drainage. Because of its characteristics the polymer supposedly reduces the required watering frequency, buffers plants from over and underwatering plus reduces the volume of rooting media needed by the plant. When used in conjunction with plug systems, the polymer helps retain moisture and decreases water stress (Aglukon Agri-Products; Congers,N.Y.) Two different polymers were used in this experiment. Viterra Planta-gel (Aglukon Agri-Products; Congers, N.Y.) and Super Slurper (University of Saskatchewan.)

After 4 weeks in agar culture media, vigorous explants of the Martin cultivar, 1.5-3.0 cm tall, were chosen. Before microcuttings were inserted into the media, the media was soaked with a nutrient solution as in experiment type A along with IBA (2.54 micromolar) and a fungicide (No-Damp) at 1 ml/l. The plantlets were then inserted into one of the appropriate media and placed in the same rooting chamber under the same conditions as in experiment type A. After 5 weeks in the rooting chamber a rooting count was performed on all trays.

## 10.3 Results

| Media | Rooting percentage |
|---|---|
| Rootrainer trays | 32% |
| Jiffy peat pellets | 17% |
| Plugs with Viterra polymer | 10% |
| Plugs with Super Slurper polymer | 10% |

As in all previous experiments the rootrainer trays exhibited the highest rooting percentage of all the different media used. However, the level of rooting in all the media was very low. Jiffy peat pellets showed a 7% higher rooting percentage than the plugs containing polymer but the Jiffy pellets were still 15% below the rootrainer trays.

The rooting percentage in the Techniculture plugs was low (10%) and uneconomical as in the previous experiments (see experiment type A). There was however, no difference in performance between the two rooting polymers used because with both polymers the rooting percentage was only 10%.

## 10.4 Discussion and Conclusions

Low levels of rooting in this experiment may be attributed to the fact that the Martin cultivar used in this experiment does not root as well as the Thiessen cultivar used in other experiments (Martin showed lower rooting percentages in experiment type A as compared to the Thiessen cultivar). Furthermore, the explants chosen for rooting out in this experiment may not have been as vigorous as explants used in other experiments due to a shortage of good material from the agar culture medium.

It was evident that the Jiffy pellets required more care than either the plugs or the rootrainers. Because of the high peat content in the Jiffy pellets, they absorb large amounts of water and are easily waterlogged. Also, the Jiffy pellets were very fragile and susceptible to damage when being moved around from the rooting chamber to the greenhouse. Furthermore,the trays provided by the Jiffy company to hold the expanded peat pellets were not space efficient and hard to carry because of their "flimsy" nature.

The rooting percentage in the plugs may have been adversely affected by the fact that the No-Damp fungicide was eventually leached out of the plugs and fungal growth began in the last stages of the experiment. Even though the rooting percentage in the plugs was uneconomical it may be assumed that the polymer used in conjunction with the plugs did increase rooting percentages. This assumption can be based upon the fact that the Martin cultivar had a lower rooting percentage in experiment type A (only 3%) when rooted in Techniculture plugs containing no polymer. From the results of this experiment the rooting polymer does not seem to increase rooting levels enough to justify its use.

The findings of this study agree with those of the previous experiments in that the best and most economical means of rooting out explants of A. alnifolia is by using rootrainer trays filled with a potting soil. As noted previously, the saskatoons growing in the Techniculture plugs had shorter roots, smaller leaves and stunted shoots. These symptoms are undesirable in the rooted material because they lead to a slow growing, non-vigorous plant which is more susceptible to stresses both in the greenhouse and upon transplanting. The explants rooted out in the Jiffy 7 peat pellets also showed similar signs of stunting which makes this system undesirable as well.

## 11. OVERALL CONCLUSIONS

In order to achieve maximum space and time efficiency in the rooting out of A. alnifolia explants it is desirable to use a rooting media such as provided by the Techniculture plug system. However, as shown by the previous experiments, there are several problems with using synthetic plugs. These problems include; restricted rooting volume, possible toxicity in the plugs,inability of saskatoon roots to penetrate the synthetic media, large changes in nutrient and moisture levels in the plugs,stunting of shoots and leaves and stems, possible poor aeration in plugs, fungal growth on the plugs as well as nutrient deficiencies in the plants grown in the plugs.

Because of the above problems, the use of plugs as a rooting media for saskatoons is not a feasible system as of yet. However, because of the many advantages in using a plug system for rooting and transplanting of micropropagated plants, further research is needed in adapting this system for use with A. alnifolia.

Jiffy peat pellets offer an intermediate rooting system between synthetic plugs and rootrainer trays. However,the Jiffy system also has several problems such as; fragility, waterlogging, "flimsy" container trays, inefficient space use as well as partial chlorosis and stunting of the saskatoons growing in them. Because of the problems involved with using the Jiffy system for rooting of saskatoons their is no practical advantage of the Jiffy peat pellets over the use of rootrainer trays.

The remaining option for rooting of micropropagated saskatoons is rootrainer trays filled with a potting soil mixture. From these experiments the use of rootrainer trays consistently showed the best rooting percentage and most vigorous plant growth. The space and time efficiency of the rootrainers is inferior to the plug system but they consistently offer the most economical rooting percentages. Because of high rooting percentages seen in the rootrainer trays, it is likely that this system will continue to be the major method used for rooting of micropropagated A. alnifolia unless higher levels of rooting can be achieved in synthetic plug systems.

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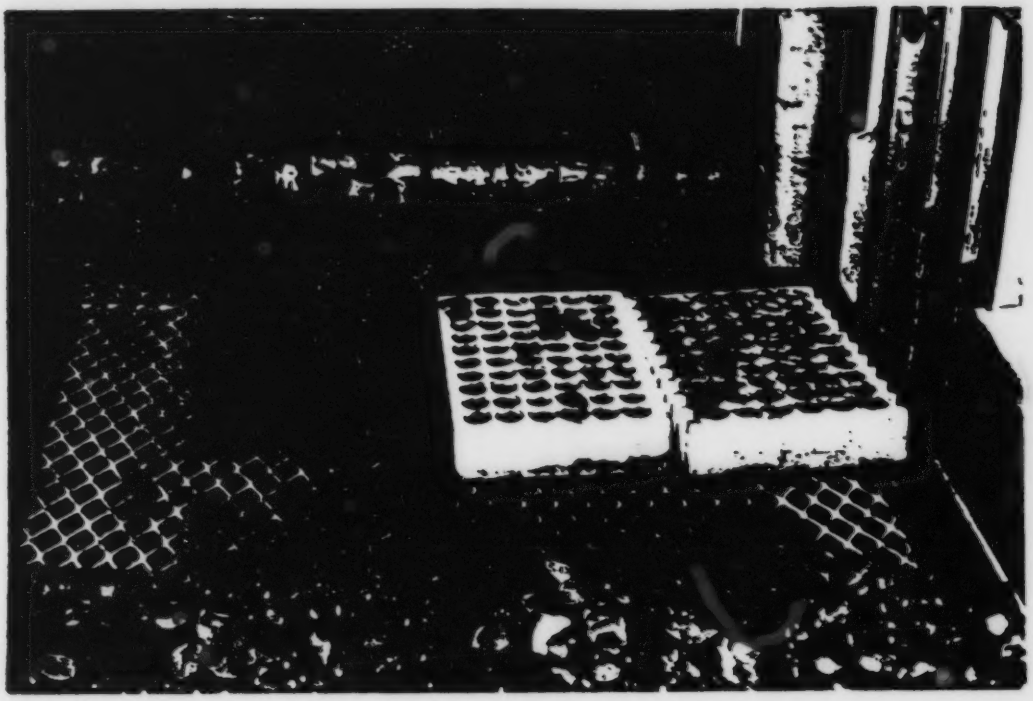

Figure 1. Rooting comparison in rootrainer trays versus Techniculture plugs.

Figure 2. Rooting comparison in rootrainer trays versus potting mixture in plug tray.

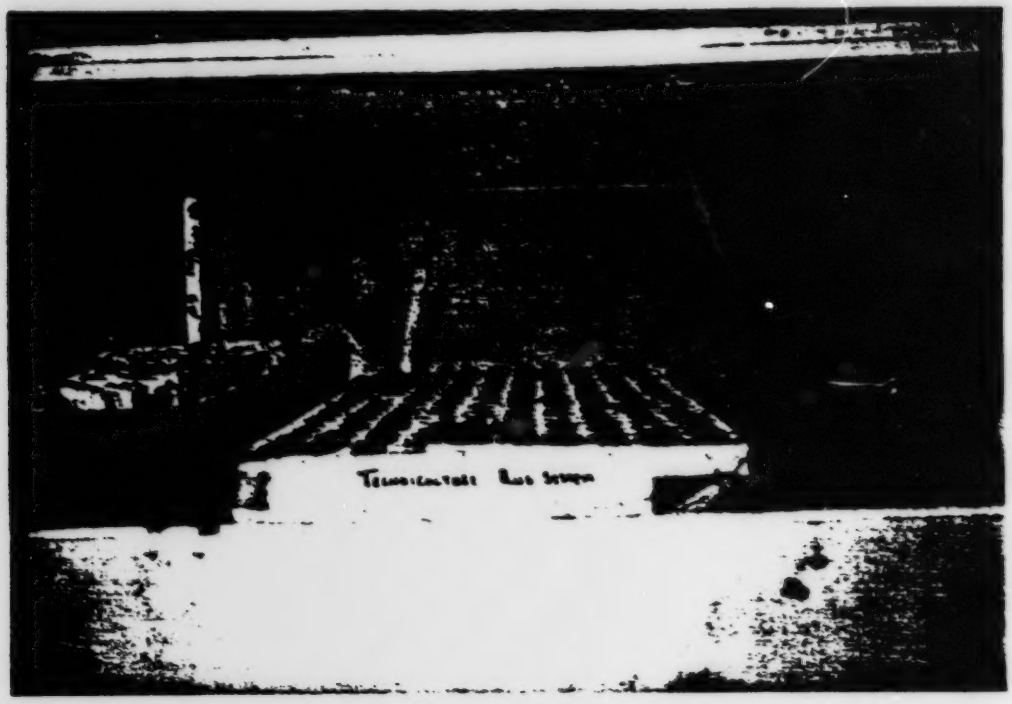

Figure 3. Saskatoons in rooting chamber showing stunting and chlorosis when grown in plugs.

Figure 4. Saskatoons in rooting chamber showing partial stunting and chlorosis when grown in Jiffy peat pellets.

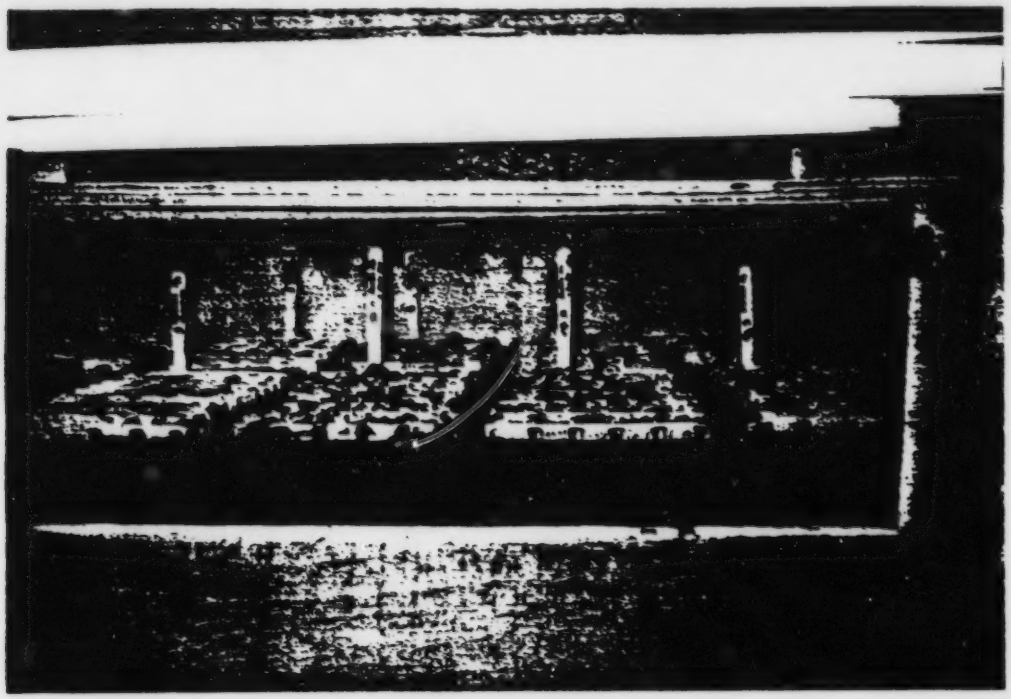

Figure 5. Saskatoons in rooting chamber growing in rootrainer trays.

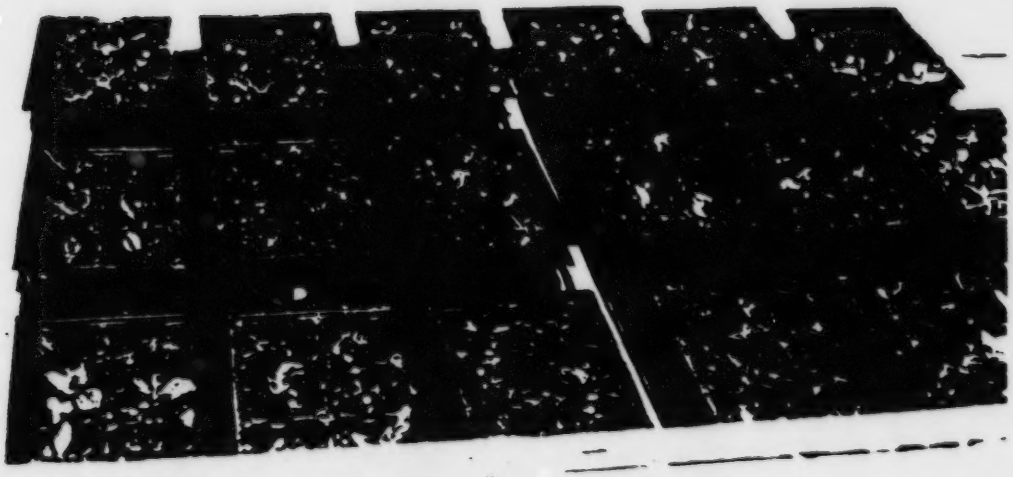

Figure 6. Small plastic containers showed initial growth but poor soil aeration resulted in 100% rooting failure.